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Cutaneous side-effects in cancer patients treated with the antiepidermal growth factor receptor antibody C225

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Summary

Background C225 is an antibody to the epidermal growth factor receptor (EGFR), and inhibits growth of various tumour cells. The antibody is currently being used as a therapeutic agent in several clinical trials of patients with carcinomas.

Objectives To determine and investigate the cutaneous side-effects in cancer patients treated with C225.

Methods We clinically examined 10 patients treated with C225, and performed immunohistochemical and *in situ* hybridization studies on skin biopsies.

Results The most common cutaneous reaction to C225 therapy was the development of an acneiform follicular eruption, which was most pronounced on the face, chest and upper back and typically manifested a week after the onset of treatment. The consistency of the morphology and timing of the clinical findings in 10 different patients following monotherapy with C225 strongly suggested a direct biological effect of the antibody. Additional dermatological side-effects included focal areas of tender paronychial inflammation of toes and fingers and small aphthous ulcers of the oral mucosa. Serial punch biopsies of chest skin before and after treatment (at 8 days) revealed two main reaction patterns: a superficial dermal inflammatory cell infiltrate surrounding hyperkeratotic and ectatic follicular infundibula, and a suppurative superficial folliculitis. In two biopsies focal intraepidermal acantholysis was found. Microbiological cultures failed to reveal an infectious aetiology. Immunohistochemical and *in situ* hybridization studies on a subset of the biopsies showed an increase in the expression of $p27^{Kip1}$ in epidermal keratinocytes after treatment with C225.

Conclusions Our findings support the concept that $p27^{Kip1}$ plays a part in the *in vivo* regulation of follicular and epidermal homeostasis by EGFR.

Key words: C225, epidermal growth factor receptor, folliculitis

C225 is an antibody to the epidermal growth factor (EGF) receptor (EGFR), and has previously been shown to block the proliferation of various cancer cells.[1–4] It is currently being investigated in several clinical trials of patients with cancer of the head and neck, breast, lung, prostate and kidney as a single therapeutic agent or in combination with other drugs.[5–7] Cutaneous side-effects are common in these patients, but have not been well characterized and the underlying mechanisms have not been investigated.

We studied the cutaneous findings of 10 patients with renal cell carcinoma who participated in a treatment protocol with C225 as a single agent. The patients were followed clinically and punch biopsies were taken at regular intervals from chest skin. We present our findings of the clinical appearance of the lesions and show the pathological findings of the biopsies.

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Materials and methods

Following written informed consent, a series of 10 patients (nine men and one woman; age range 47–76 years, mean 58·4) enrolled in a non-randomized phase

II clinical trial of C225 antibody therapy for metastatic renal cell carcinoma at the Memorial Sloan–Kettering Cancer Center (New York) were studied with baseline and weekly dermatological evaluation and photography. Patients who had received prior chemotherapy or immunotherapy were excluded from the study. Study participants received C225 by intravenous infusion weekly at an initial loading dose of 500 mg m^{-2} followed by seven consecutive weekly doses at 250 mg m^{-2}. A baseline questionnaire documented Fitzpatrick skin type, past or recent history of acne, and all topical and systemic medications being used at time of study entry.

The severity of the cutaneous reactions was graded at weekly follow-up visits as: grade 1, asymptomatic macular or papular erythematous eruption in an acneiform distribution; grade 2, same as 1, but with symptoms such as pruritus; grade 3, extension of the eruption beyond the acneiform distribution of head, chest and back or the development of confluent lesions, painful lesions, or minor ulceration; grade 4, exfoliative or ulcerating dermatitis.

Surveillance bacterial cultures of forehead skin were performed at baseline and day 8. Following a gentle cleansing of the forehead, a sterile PVM/MA copolymer-impregnated strip (Chesebrough-Pond's, NJ, U.S.A.) was applied to the skin for 5 min and the stratum corneum and follicular contents stripped from the skin. The strip contents were suspended in an aliquot of bacterial medium and plated on bacterial and fungal media.

Serial 4-mm punch biopsies of chest skin were performed immediately prior to the initial infusion, 1 h following completion of the initial infusion, and on day 8 of therapy. In addition, several of the patients underwent a skin biopsy on day 3 or 4 of therapy. The biopsies were kept fresh in saline. Upon arrival in the pathology laboratory (Memorial Sloan–Kettering Cancer Center, New York), the biopsies were split in half. One half was frozen, the other routinely processed, i.e. fixed in 10% formalin and embedded in paraffin. Multiple haematoxylin and eosin-stained sections per biopsy were examined and Gram as well as periodic acid-Schiff-D stains were obtained to assess the presence or absence of bacteria and fungi. For cases that lacked a follicle or had only minimal inflammation on initial sectioning, the tissue block was extensively step-sectioned in search of a follicle and/or more prominent inflammation.

Immunohistochemistry was performed on biopsies from four patients whose tissue block contained sufficient material for such an analysis. The avidin–biotin method was used with the monoclonal antibody against $p27^{Kip1}$ (Ab-2; Oncogene Science, Inc., Boston, MA, U.S.A.; 0·1 μg mL^{-1} final concentration) as previously described,[8,9] and UCHL1 (pan-T-cell marker; Dako; 1 : 200). Endogenous peroxidase was suppressed by a 20-min incubation with 1% H_2O_2. Labelling of the secondary antibody was performed with an avidin–biotin complex using a biotinylated horse antimouse secondary (Vector, Burlingame, CA, U.S.A.; 1 : 200 dilution) and diaminobenzidine tetrahydrochloride (Biogenex, San Ramon, CA, U.S.A.) as a chromogen. The number of stained nuclei was counted per high-power microscope field (HPF; × 40 objective). Three HPFs were examined per biopsy.

In situ hybridization studies for $p27^{Kip1}$ mRNA expression were carried out as previously described.[8,9] Digoxigenin-labelled probes were used and 1 μg of recombinant plasmid pCRTMII (Invitrogen Corp., San Diego, CA, U.S.A.), containing the full-length human p27KIP1 gene (a gift from Dr M.Pagano, New York University School of Medicine), was linearized by *Bam*HI and *Xba*I to generate anti-sense and sense transcripts. Tissue sections were rinsed in water and phosphate-buffered saline (PBS) for 10 min. The slides were digested with proteinase K (50 μg mL^{-1}) for 18 min at 37 °C in PBS and postfixed at 4 °C in a freshly prepared solution of 4% paraformaldehyde in PBS for 5 min. Prehybridization was for 30 min at 45 °C in 50% formamide and 2 × standard saline citrate (SSC). The hybridization buffer contained 50% (v/v) deionized formamide, 10% dextran sulphate (50% stock solution), 2 × SSC (20 × stock solution), 1% sodium dodecyl sulphate (10% stock solution) and herring sperm DNA (0·25 μg mL^{-1}). Hybridization was performed overnight at 45 °C by applying digoxigenin-labelled RNA probe (10 pmol L^{-1} in 50 μL of hybridization buffer per section) under a coverslip. The coverslips were removed, and the slides were washed in prewarmed 2 × SSC for 20 min at 60 °C twice, followed by washes in prewarmed 0·5 × SSC and 0·021 × SSC each at 60 °C for 20 min. After these washes, the slides were incubated in normal sheep serum diluted in buffer at pH 7·5 and successively in the same buffer with antidigoxigenin antibody conjugated to alkaline phosphatase (Roche Diagnostics, IN, USA) at a dilution of 1 : 1500 for 1 h at room temperature. The visualization was accomplished by use of nitroblue tetrazolium 5-bromo-4-chloro-3-indolyl phosphate. The slides were counterstained with methyl green and mounted.

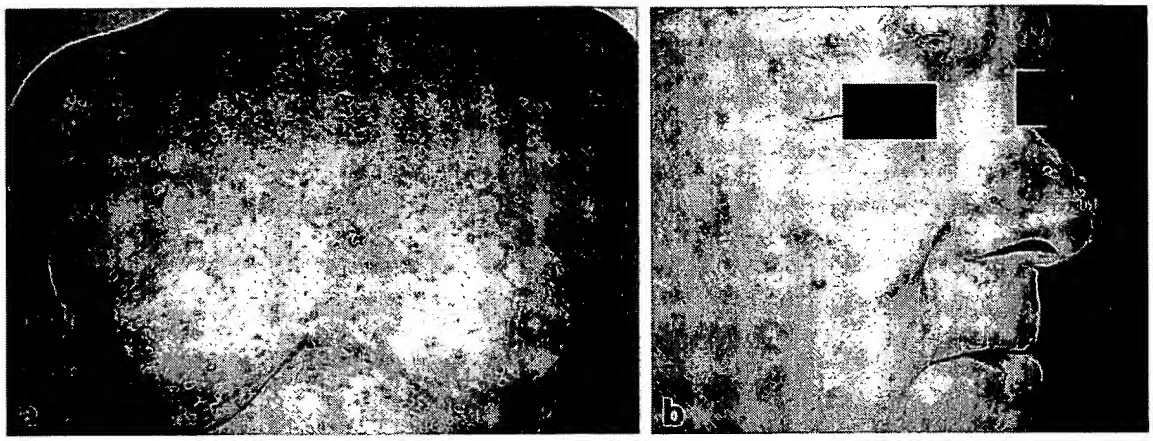

Figure 1. Clinical spectrum of the follicular rash. (a) Involvement of the skin of the chest (patient 3). (b) Involvement of the skin of the face (patient 2).

Results

Clinical findings

The most common side-effect of C225 therapy was a follicular rash, which commonly began in an acneiform distribution (face, scalp, chest and upper back) approximately 1 week into therapy (Fig. 1). It developed in all 10 patients. The time course of the clinical severity of the rash is depicted in Figure 2. There was no apparent association between severity of the rash and skin type or history of acne. Empirical use of topical and systemic antibiotics as well as topical steroids was not associated with consistent clinical improvement of the rash.

Another common dermatological manifestation in our patients was pain, tenderness and fissuring of the distal finger tufts that occurred in varying degrees in all of the patients. Five patients developed paronychial inflammation with associated swelling of the lateral nail folds of toes and fingers (Fig. 3). The digits most commonly affected were the great toes and thumbs. In one patient the middle finger was affected as well. In several instances the lateral nail fold swelling was associated with friable pyogenic granuloma-like changes that bled with minimal trauma. There was no preceding history of ingrown nails. Neither *Candida* nor bacteria were found on culture at the onset of these lesions. *Staphylococcus aureus* was cultured from some lesions, and persisted despite antibiotic therapy. Greatest symptomatic relief was achieved with soaks and cushioning of affected areas.

Two patients reported minimally symptomatic sores of the oral mucosa during therapy. They were found to have a few 2–3 mm intraoral aphthous ulcers. Herpesvirus direct immunofluorescence and culture were negative in these patients.

Histological findings

The histological findings in serial punch biopsies performed on chest skin from nine patients treated

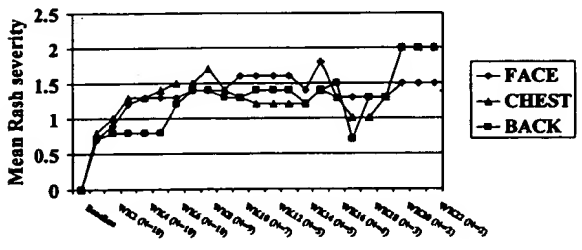

Figure 2. Severity of the rash over time by site.

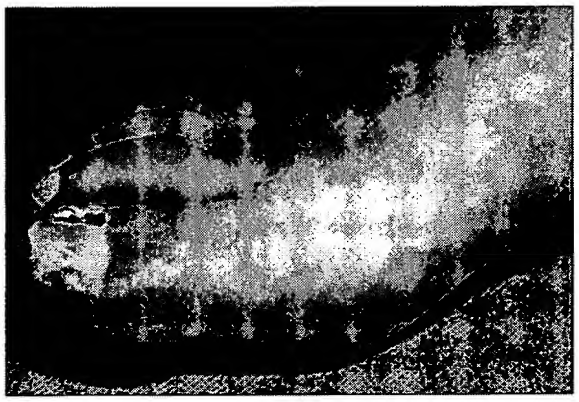

Figure 3. Paronychial swelling and friability of the lateral nail fold of the great toe (patient 2).

Table 1. Summary of histological findings

Patient	Day of treatment	Histological findings
1	1	Normal skin
	8	Suppurative folliculitis
2	1	Normal skin
	3	Superficial perifolliculitis
	8	Suppurative superficial folliculitis
3	1	Normal skin
	4	Suppurative folliculitis
	8	Suppurative folliculitis
4	1	Normal skin
	8	Perivascular dermatitis (no follicle present)
5	1	Normal skin
	4	Suppurative folliculitis; focal intraepidermal acantholysis
	8	Suppurative folliculitis
6	1	Normal skin
	8	Focal intraepidermal acantholysis and mild perifolliculitis
7	1	Normal skin
	8	Superficial perifolliculitis
8	1	Normal skin
	8	Superficial perifolliculitis
9	1	Normal skin
	8	Superficial perifolliculitis

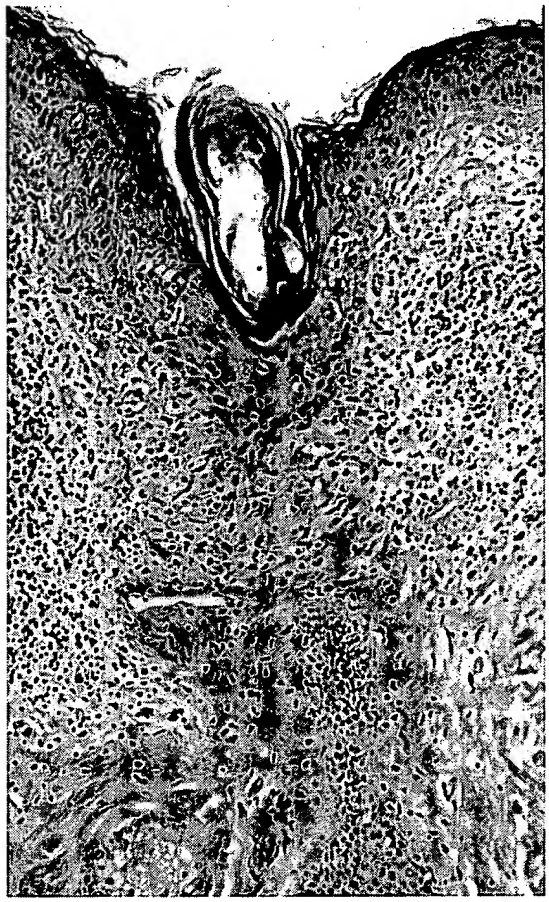

Figure 4. Superficial dermal infiltrate of lymphocytes surrounding a hyperkeratotic follicular infundibulum (haematoxylin and eosin; original magnification ×200).

with C225 are summarized in Table 1. Before treatment with C225 was initiated, a control biopsy of normal skin was obtained, which was unremarkable in all cases, except for the presence of rare incidental *Pityrosporum* organisms in the stratum corneum of three patients. The subsequent biopsies showed variable inflammatory changes. The earliest findings included an infiltrate of T lymphocytes (immunoreactive for CD45RO; not shown) surrounding the follicular infundibulum (Fig. 4). After 1 week of treatment, four patients had a superficial perifolliculitis involving hyperkeratotic and ectatic follicular infundibula (Table 1), four had a florid suppurative folliculitis (Fig. 5), and two showed focal intraepidermal acantholysis in association with a sparse neutrophilic infiltrate involving the terminal portion of the sweat duct (Fig. 6). In one patient, the biopsy obtained at day 8 lacked a follicle upon serial sectioning. Thus, evaluation of follicular or perifollicular inflammation was not possible in this case.

Table 2. Immunoreactivity for $p27^{Kip1}$

Patient	No. of stained nuclei per high-power microscope field	
	Prior to treatment	Day 8 of treatment
1	6	20
2	8	25
3	7	32
5	12	35

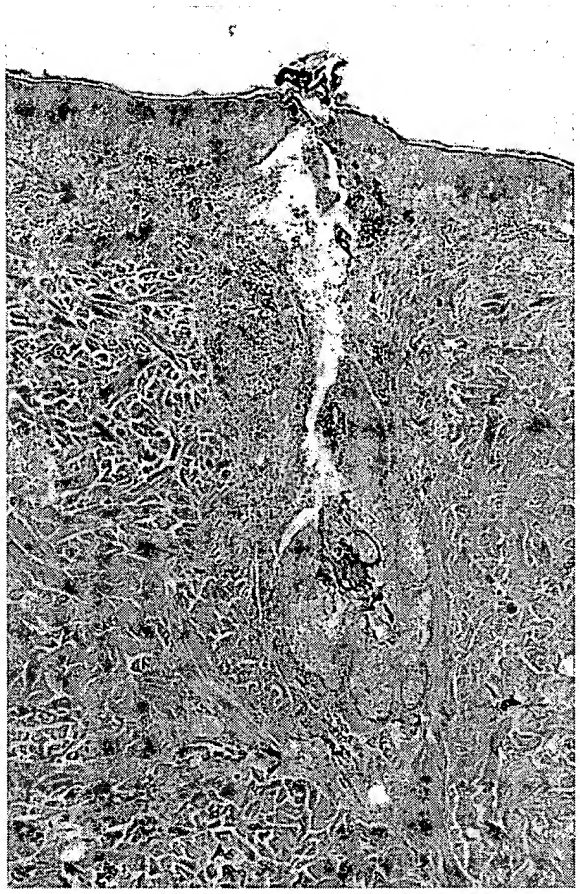

Figure 5. Suppurative superficial folliculitis. A hair follicle is involved by a florid mixed inflammatory cell infiltrate and rupture of the epithelial lining is present (haematoxylin and eosin; original magnification × 40).

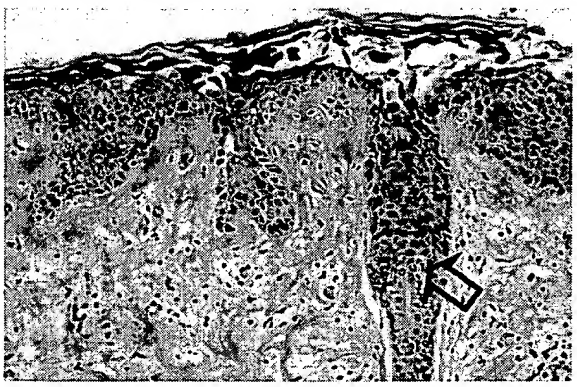

Figure 6. Intraepidermal acantholysis overlying the terminal portion of a sweat duct, which is focally involved by a neutrophilic infiltrate (arrow) (haematoxylin and eosin; original magnification ×100).

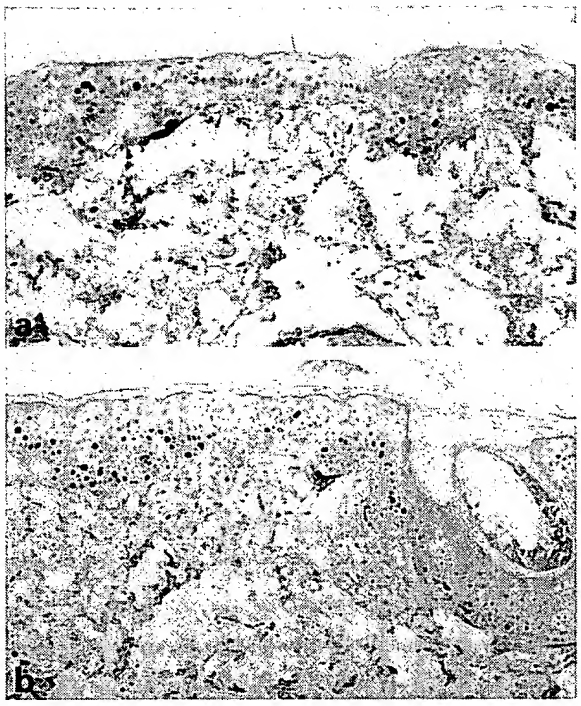

Figure 7. Immunoreactivity for $p27^{Kip1}$ in epidermal keratinocytes. Fewer nuclei are immunopositive prior to treatment with C225 (a) than 1 week after treatment (b). (Original magnification × 40)

Immunohistochemical findings

The expression of the negative growth regulator $p27^{Kip1}$ was analysed by immunohistochemistry in four patients whose biopsies contained sufficient tissue for analysis. The results are summarized in Table 2. At day 8 of treatment with C225, a three- to fourfold increase in the number of immunolabelled interfollicular epidermal keratinocytes was found. While in normal epidermis only occasional basilar keratinocytes stained positive (Fig. 7a), keratinocytes at all cell layers of the epidermis stained for $p27^{Kip1}$ in skin of treated patients (Fig. 7b).

In situ *hybridization*

In those cases in which the expression of $p27^{Kip1}$ was analysed by immunohistochemistry, *in situ* hybridization studies were performed in parallel. Labelling was most pronounced in basilar and suprabasilar keratinocytes. There was an approximately twofold increase in cytoplasmic labelling for p27 mRNA in epidermal keratinocytes of treated skin compared with untreated skin (not shown).

Microbiological cultures

Surveillance cultures of facial skin failed to reveal any consistent significant changes in the cutaneous microflora. Neither *Candida* nor bacteria were found on culture at the onset of these lesions, although *S. aureus* was cultured from some persistent lesions.

Discussion

This study describes the cutaneous reactions seen in 10 patients with renal cell carcinoma treated with C225. We focused our analysis on the most common reaction, which was an acneiform follicular eruption, histologically characterized by a lymphocytic perifolliculitis or suppurative superficial folliculitis. The absence of an apparent infectious aetiology, the consistency of the clinical findings across patients, as well as the absence of another drug or plausible aetiology suggest C225 as the causative agent of this follicular dermatitis. Additional indirect evidence for this hypothesis comes from the clinical observation that we have seen a similar acneiform eruption in a patient treated with 4-(3-chloro-4-fluorophenylamino)-7-methoxy-6-(3-(4-morpholinyl) propoxy)-quinazoline, which is known to block EGFR-dependent activation of tyrosine kinase activity.[10]

EGFR is expressed in the epidermis, the sweat gland apparatus and hair follicle epithelium.[11–14] Previous experimental studies have demonstrated a central role of the EGFR in the normal differentiation and development of the hair follicle.[15–21] Mice harbouring a targeted disruption of the EGFR allele exhibit a disorganized hair follicle phenotype.[15–21] EGF has pleiotropic effects on keratinocytes. Administration of EGF to newborn mice delays hair follicle development, decreases the rate of growth, and reduces the hair diameter.[22,23] While it slows the growth and affects the differentiation of hair follicles, EGF is a potent mitogen on cultured keratinocytes and leads to epidermal hyperplasia and hyperkeratosis.[24–27]

Valuable information on the role of growth factors in the homeostasis of the pilosebaceous unit has been gained from *in vitro* studies.[28–34] *In vitro* models have demonstrated that EGF and transforming growth factor (TGF)-α are involved in the triggering of anagen and catagen.[28,29] A critical role for EGF and TGF-α in regulating the differentiation of sebocytes and keratinocytes of the human pilosebaceous infundibulum has been established using organ cultures.[30,31] EGF and TGF-α, however, are not the only growth factors relevant for the biology of hair follicles. Several other cytokines, such as insulin-like growth factor, interleukins and tumour necrosis factor-α are also known to play a part.[32–34]

The effects of C225 on the skin are likely to be a direct consequence of its interference with the signal pathway of EGFR. Among potential candidates of proteins involved in the postreceptor effects of C225, $p27^{Kip1}$ merits attention. It is a negative growth regulator that binds to and inactivates cyclin-dependent kinase-2, thereby leading to cell cycle arrest in G_1.[35,36] Several *in vitro* studies on various tumour cells have shown that upregulation of $p27^{Kip1}$ plays a crucial part in growth inhibition by C225.[37–42] Therefore, we examined the effect of C225 treatment on $p27^{Kip1}$ expression in the epidermis by immunohistochemistry and *in situ* hybridization. A three- to fourfold increase in $p27^{Kip1}$-positive keratinocyte nuclei was observed by the eighth day of treatment, which was accompanied by an increase in $p27^{Kip1}$ mRNA levels. Although it is difficult to draw reliable conclusions from a small number of cases (four patients) in which sufficient lesional tissue was left for analysis, the consistency of increased $p27^{Kip1}$ expression in all cases suggests that that $p27^{Kip1}$ could play a part in mediating the effects of C225 *in vivo*. It also indicates that C225 probably has a direct effect on keratinocytes. Such a direct effect of C225 on target cells was recently demonstrated for mucosal epithelium in a patient with Menetrier's disease.[43] Analysis of protein extracts from gastric fundic biopsies suggested that treatment with C225 resulted in lower concentrations of mitogen-associated protein kinase and a decrease in the proliferative index of gastric glandular epithelium.[43]

C225 probably interferes with the EGFR signal pathway of epidermal and skin adnexal epithelium. The upregulation of $p27^{Kip1}$ observed in our study probably leads to some impairment of cell growth and associated altered differentiation. However, the precise mechanism by which altered levels of $p27^{Kip1}$ contribute to an acneiform eruption remains elusive at the current time. Further experiments are needed to explore the role of $p27^{Kip1}$ in follicular growth and differentiation. It is possible that altered levels of $p27^{Kip1}$ are solely a marker for the effect of C225 and are not causally linked to the acneiform eruption. Other proteins in the signal pathway of EGFR, such as mitogen-associated protein kinase, need to be studied for their role in follicular homeostasis, as they may provide clues to the mechanism of C225 as well as our understanding of the formation of acne.

It is also difficult to establish a causal link between C225 and the presence of an inflammatory reaction. One possible explanation for the inflammatory infiltrate in the biopsies with suppurative inflammation is that it occurs in response to the follicular rupture observed in some specimens. Such a mechanical rupture may have its origin from an effect of C225 on follicular growth and differentiation, which may lead to excessive hyperkeratosis, follicular plugging and subsequent obstruction of the follicular ostium, similar to processes known to occur in acne. A perifollicular inflammatory cell infiltrate in the presence of an intact follicle is more difficult to explain. It is possible that an alteration of follicular growth and differentiation affects the microflora of the skin, especially within the stratum corneum of the follicular infundibulum, which may subsequently elicit an inflammatory reaction. Such a presumed alteration of the cutaneous microflora is not supported by the results from our limited microbiological studies and special stains for micro-organisms. It is, however, conceivable that the inflammatory reaction is more directly related to C225. The mere presence of an antibody on the surface of follicular epithelial cells may directly elicit an inflammatory reaction, which in turn could lead to follicular rupture. Experimental studies are needed to investigate the cause of the inflammation further.

The sparse neutrophilic inflammation involving the terminal portion of the eccrine duct, which was accompanied by focal intraepidermal acantholysis, represents an unusual histological reaction pattern (Fig. 6). We have no explanation for the mechanism of this phenomenon. However, as EGFR is strongly expressed in sweat duct epithelium,[14] alterations in the pathophysiology of eccrine ducts of patients treated with antibodies against EGFR are not a surprise.

The paronychial changes observed in our patients are similar to those previously described with systemic retinoid and antiretroviral therapy.[44–46] The specific mechanisms by which these lesions are induced by retinoids and antiretroviral agents are unknown. Our observations suggest a possible role for EGFR in the induction of these lesions.

In summary, we describe the cutaneous side-effects seen in 10 cancer patients treated with C225. We document the histological findings of the most common eruption, which was an acneiform follicular or perifollicular dermatitis. We believe that C225 is the cause of this eruption. To our knowledge, this represents the first report of cutaneous side-effects related to the treatment of patients with a humanized monoclonal antibody. Our results show that treatment with C225 results in upregulation of $p27^{Kip1}$ in epidermal keratinocytes, which indicates that alteration of $p27^{Kip1}$ may be a mechanism by which C225 can affect follicular and epidermal homeostasis. The similarity of the follicular eruption described herein to acne suggests that further investigations into the disturbance of the EGFR pathway and cell cycle regulation in keratinocytes may be relevant to our understanding of acne and acneiform skin eruptions.

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